Conformational Variability of Soluble Cellulose Oligomers

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Cellulose occurs naturally as microfibrils in the cell walls of higher plants and is also produced by some bacteria, algae, fungi, and sea animals. Two distinct crystal phases, Ia and Ib, are found in nature (collectively called cellulose I) in proportions that depend on the origin of the cellulose. For many applications, raw cellulosic material is pretreated or processed so that the cell wall architecture is disrupted and so that the cellulose crystal structure is transformed from cellulose I to other crystal phases that have improved properties for their specific application. These different crystal phases have very different properties. In particular, their different susceptibilities to digestion by cellulases is of importance in biomass conversion, i.e., the conversion of biomass into sugars that can be used in the production of biofuels. As part of our ongoing research in biomass conversion, we have been studying the molecular aspects, both structural and dynamical, of the formation of the different cellulose polymorphs and how these molecular aspects are also important for the interaction of the different polymorphs with cellulases [1].

Our most recent work concerns individual molecules of cellulose oligomers in aqueous solution [2]. Several questions remain unanswered regarding the conformational dynamics of these short chains. For example, do the glycosidic linkages have greater flexibility when freed from the restrictions imposed by the crystal environment? How do the local conformational propensities change with increasing degree of polymerization? Which internal motions are the most sensitive to thermal perturbation? It is useful to address these questions for different lengths of cellulose chains because during enzymatic depolymerization, varying lengths of cellulose chain fragments are generated. Short glucose oligomers produced by these enzymes can hinder the overall efficiency of biomass conversion to sugar. These effects include product inhibition, competition with substrates of other enzymes, and interference with the synergetic action of cellulases by adsorption back onto the cellulose microfibril. Therefore, structural and dynamical characterization of different oligomer sizes and their relative stabilities needs to be taken into consideration when designing enzymatic cocktails.

In this study, all-atom replica exchange molecular dynamics (REMD) simulations have been used to probe the molecular aspects of the soluble oligosaccharides. The all-atom details of the simulations ensure that intra-chain and solvent hydrogen bonding, hydrophobicity and solvent ordering, and dynamics are properly taken into account. This work is one of only a few theoretical studies of the conformational dynamics of varying lengths of carbohydrate chains. Therefore, our effort will also serve as a measure of the quality of the force field and the capability of the chosen theoretical method (REMD) to provide efficient sampling of carbohydrates. To our knowledge, this work stands out as the first REMD study on polysaccharides in an aqueous environment. The use of REMD and long simulation times should provide more thorough sampling than in previous studies, and the multiple temperatures considered add considerable new information.

Several interesting properties are obtained from REMD simulations. As chain length is increased, the conformations of the oligomers become more rigid and likely to form intra-chain hydrogen bonds, like those found in crystals. Several other conformations and hydrogen bonding patterns distinguish these short cellulose chains from those in cellulose crystals. These studies have also addressed the key role played by solvent on shifting the conformational preferences of the oligosaccharides with respect to vacuum and crystals. Correlation between pyranose ring flipping and the conformation of the 1,4-glycosidic bond was observed. Comparisons to known measurements of oligomers and crystals are made whenever possible.

Finally, we have shown that cellulose oligomers have different properties from chains in the crystalline forms of cellulose. The results presented here may be relevant for understanding the properties of cellulose fragments during degradation by cellulases. During this enzymatic process, cellulose chains are frayed and extracted from the crystalline environments. They can potentially form alternate conformations with cellulase or when transferred from the crystalline phase to the aqueous phase.

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[1] T. Shen, S. Gnanakaran, *Biophys. J.* **96,** 3032 (2009).
[2] T. Shen, et al., *J. Am. Chem. Soc.* **131,** 14786 (2009).

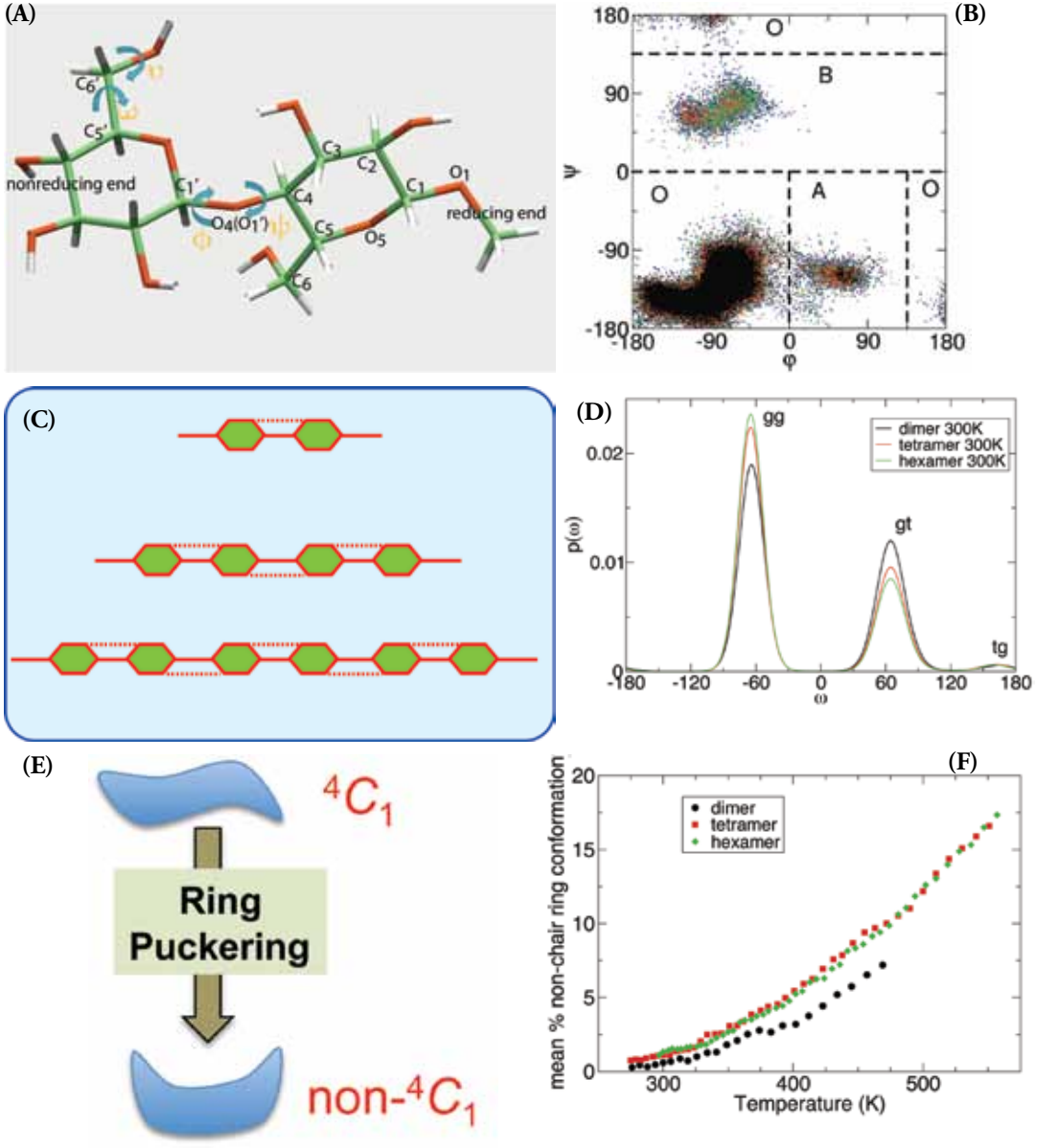

Fig. 1. (A) Atomistic structure of methyl beta-D-cellobioside, representing the dimer. Torsional angles of glycosidic backbone and hydroxymethyl side chain are marked. (B) The distribution of the critical torsion angle for the glycosidic linkage in dimer is shown. The division of backbone torsion conformational space into three discrete regions O, A, and B is also displayed. (C) Schematic structure of dimer, tetramer, and hexamer considered in the simulation study. (D) The populations of the torsion angles of the hydroxymethyl side chain are plotted in terms of two-letter code that is used to describe the three conformations (gg,gt, and tg). (E) A schematic picture illustrating the possible sugar ring puckering in cellulose. Sugar rings are in chain-like ($^4C_1$) conformation in cellulose crystals. (F) This plot captures the sugar ring puckering in cellulose oligomers. The mean percentage of glucopyranose rings in strongly distorted non-$^4C_1$ conformations is shown as a function of temperature.

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